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Regional Localization of Two MRX Genes to Xq28 (MRX28) and to Xp11.4–Xp22.12 (MRX33)

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Two genes responsible for a nonspecific form of X-linked mental retardation (MRX28 and MRX33) were localized by linkage analysis with 40 highly polymorphic DNA markers situated along the entire the X chromosome. In family 1, the gene could be mapped within a 14-cM interval at Xq28, distal to the recombining marker DXS1113 (MRX28). The maximum LOD score was 2.75, with DXS52 at ϕ = .0. In family 2, the gene was localized within a 30-cM interval at Xp11.4–22.12 between the recombining markers DXS365 and MAOB, including the DMD gene (MRX33). Maximum LOD scores of 2.82 were obtained with markers DMD-STR49, DMD-DysII, CYBB, and DXS1068.

KEY WORDS: gene localization, mental retardation, MRX, Xq28, Xp11.4–22.12, linkage analysis

INTRODUCTION

X-linked mental retardation (XLMR) is a common condition affecting approximately 1 in 550 males, with the fragile X (fra(X)) syndrome accounting for approximately 15% of cases [Gendrot et al., 1994; Turner et al., 1996]. Linkage analysis using polymorphic markers in large XLMR families enables regional localization of MRX gene loci. A revised nomenclature for MRX genes allocates a number to every MRX family that has been investigated under specific guidelines [Mulley et al., 1992]. At present, genetic mapping is the only way to discriminate XLMR families, although syndrome manifestations are sometimes available for differentiation. Initially, 4 nonoverlapping MRX intervals with a most prominent one in the pericentromeric region could be defined on the X chromosome [Neri et al., 1994]. Because highly polymorphic minisatellite markers have been made available [Gyapay et al., 1994], better resolution for the genetic intervals have been obtained, which has led to the definition of actually more than 50 MRX loci that can be subdivided into at least 10 nonoverlapping genetic intervals (H. Lubs, personal communication).

We report on linkage analysis in 2 families with XLMR without further consistent clinical symptoms and without heterozygote manifestation.

CLINICAL REPORT

Family 1 (MRX28)

This family (Fig. 1A) was identified in 1989 when it was referred for genetic counseling because of a suspected fra(X) syndrome in the affected males, which was excluded by cytogenetic and molecular analysis.

IV-7. He was delivered after 7 months of a normal pregnancy, with a birth weight of approximately 2,400 g; he had to be nursed in an incubator for several days. There were no further neonatal problems. His development was delayed; he learned to walk at the age of 3 years and began to speak single words at the age of 4. His general health is good. He attended a special school for the moderately intellectually handicapped, where he achieved normal speech and learned to write single words. At the time of clinical investigation, he was 42 years old, with a weight of 70 kg, height of 168 cm, and head circumference of 56 cm, which is normal. He had brachycephaly, a long face, short palpebral fissures, a broad globular nose, inverted upper lip and an everted lower lip, slightly flexed finger joints, and a kyphosis.

V-7. He was delivered after 7 months of an uncomplicated pregnancy, with a birth weight of 2,500 g; he also had to be nursed in an incubator for a couple of days. There were no further neonatal problems or any other recognized medical problems during infancy. He started to walk at 20 months and speak at approximately 2 years of age. At 6 years old, he attended a normal primary school. From age 8 to 15 years, he attended a school for the moderately intellectually handicapped. He achieved reading and writing abilities and is now employed in a sheltered workshop. He presents

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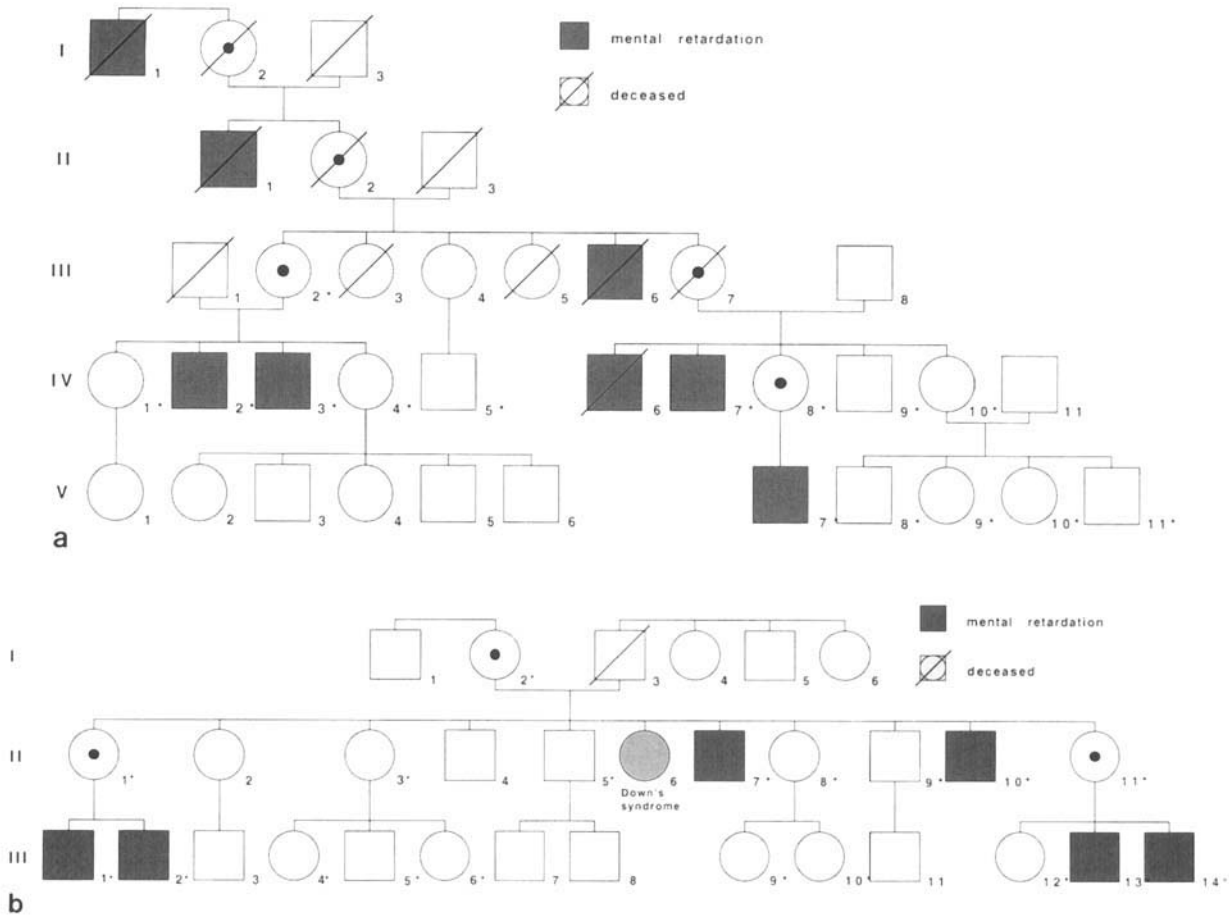

Fig. 1. Pedigrees of kindreds MRX28 (**A**) and MRX33 (**B**), showing affected individuals as shaded boxes and carriers as circles with dot. Individuals for whom DNA was available are indicated by an asterisk

as a mildly retarded person with a friendly personality. Presently, he has a height of 170 cm, a weight of 74 kg, and a head circumference of 55 cm. He also has brachycephaly, a long face, a right palpebral fissure that is shorter than the left, a broad nose with a pointed tip, an inverted upper lip, and an everted lower lip.

IV-2. He was born at term. His psychomotor development was delayed, and he walked after the age of 2 years. He was slow to acquire speech and communicates now in short simple sentences. From 6 to 15 years of age, he attended a school for the developmentally disabled. Presently, he lives in a hostel for intellectually handicapped and daily attends a sheltered workshop. His behavior is difficult; he is aggressive and easily upset. He does not show any minor anomalies except for large ears.

IV-3. He was delivered at term after an uncomplicated pregnancy. His development was normal. At 7 years of age, he was sent to a normal primary school, where he needed special help. From 10 to 15 years of age, he attended the same special school as his brother, but he was much more capable. He has no behavior difficulties or minor anomalies.

Family 1 (MRX28) segregates a nonsyndromic form of XLMR; the affected males do not show any consistent syndromal anomalies. The degree of mental impairment is variable, ranging from moderate to mild mental retardation. Obligate carrier women are not affected.

Family 2 (MRX33)

The second family (Fig. 1B) was referred because of suspected fra(X) syndrome in the affected males, which was excluded. The obligate carrier women do not show any manifestations. Three of the affected males underwent clinical examination in a department for clinical genetics; on 2 of the affected males, only anamnestic data were available.

II-7. No exact data were available about his development except for delayed acquisition of speech. He never attended school and now lives in a hostel for the intellectually handicapped. At the time of the study, he was 41 years old, and appeared slightly overweight, with a weight of 80 kg and a height of 174 cm. His head circumference was 57.5 cm. His face appears disproportionately large for the head circumference, and he is

brachycephalic. He has full lips, long ears (7.2 and 7.5 cm), short broad fingers, and strong male body hair distribution. His testicular volume (25 ml) is within the upper normal range. He has diabetes mellitus type II.

II-10. No exact data were available about his developmental milestones during infancy, except for delayed speech acquisition. Like his brother, he never attended school and now lives in a hostel for the intellectually handicapped. He was 32 years old at the time of the clinical examination. He also is overweight (85 kg, 172 cm) and has the same appearance as his brother. His head circumference is 57 cm; he appears brachycephalic, like his brother. He has also long ears (7.2 and 7.5 cm), and his fingers are short and broad. His testicular volume is over 25 ml on the right and aproximately 20 ml on the left. He also has diabetes mellitus type II.

III-1. He was delivered 3 weeks before term; he stuttered during childhood and was clumsy. He attended a school for intellectually handicapped for 8 years, where he achieved reading and writing abilities. At the time of the examination, he was 23 years old and did not have the minor anomalies and long ears like II-7 and II-10.

III-2. The brother of III-1 was not available for examination. According to anamnestic data and photographs, he does not have any minor anomaly. We did not have much information about his intellectual abilities except that he attended the same school as his brother.

III-13. Only anamnestic data were available. He was delivered at term, and his motor development was mildly retarded (sitting by 10 months and walking by 18 months). Cognitive development was quite delayed; he learned to use single vowels for communication at 3 years of age and has not progressed in speech acquisition. He is very hyperactive and now attends a therapeutic kindergarten. According to anamnestic data and photographs, he does not have any minor anomaly.

III-14. He is similar to his brother but with mild motoric retardation. He was more capable in the acquisition of speech, and he learned to use single words at 3 years of age but without progress. He does not have any syndromal manifestations.

In family 2 (MRX33), affected males do not have any consistent physical findings. All have mild retardation of motor development and more severe retardation in intellectual abilities, especially in the acquisition of speech. As in family 1, the affected males show a variable degree of mental impairment, and a correlation may exist between the severity of mental retardation and the expression of physical anomalies.

DNA AND CYTOGENETIC AND LINKAGE ANALYSIS

DNA and Cytogenetic Analysis

DNA was extracted from blood leukocytes as described by Miller et al. [1988]. DNA samples of the affected males and obligate carriers were tested by using Southern blot analysis with the FRAXA-specific probe pP2 [Oostra and Verkerk, 1992] and the FRAXE-specific probe OxE20 [Knight et al., 1993], supplemented with a polymerase chain reaction (PCR) analysis of the CGG repeat in the FMR-1 gene [Fu et al., 1991] and a PCR analysis of the GCC repeat in the FRAXE locus [Knight et al., 1993].

For the linkage analysis, 40 polymorphic markers along the entire length of the X chromosome were tested (Table I). Most of the markers used were short tandem repeat (STR) sequences. The PCR reaction was carried out in a total volume of 50 μl by using approximately 500 ng of DNA in 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.01% gelatin, 1 U Taq-Polymerase (Amersham), and 20–50 pmol of each primer. The cycle conditions used for amplification by PCR were 5 min at 95°C for denaturation, 30 cycles with 1 min at 95°C, 1 min for each STR-specific annealing temerature, and 1 min at 72°C. All amplifications were finished by a prolonged extension step (5 min) at 72°C. Allelic variations in STR sequences were demonstrated by electrophoresis on a 6% acrylamide gel under denaturating conditions.

TABLE I. Polymorphic Markers on the X Chromosome[a]

Localization	Locus	Probe or PCR	Enzyme
Xp22.32	DXS996	(CA)n	
1Xp22.31	KAL5′	(CA)n	
Xp22.12	DXS987	(CA)n	
Xp22.11	DXS989	(CA)n	
Xp22.13	DXS365	(CA)n	
Xp21.2	STR49	(CA)n	
Xp21.2	STR45	(CA)n	
Xp21.2	DysII	(CA)n	
Xp21.1	CYBB	(CA)n	
Xp11.4	DXS1068	(CA)n	
Xp11.3	MAOB	(CA)n	
Xp11.22	DXS255	M27β	EcoRI/PstI
Xp11.22	DXS988	(CA)n	
Xq12	AR	(CAG)n	
Xq21.1	DXS453	(CA)n	
Xq21.31	DXS990	(CA)n	
Xq21.33	DXS458	(CA)n	
Xq22.1	DXS454	(CA)n	
Xq22.1	DXS178	(CA)n	
Xq22.3	DXS456	(CA)n	
Xq22.3	COL4A5	(CA)n	
Xq23	DXS1001	(CA)n	
Xq25	DXS692	(CA)n	
Xq26.1	DXS86		BglII
Xq26.2	CD40L	(CA)n	
Xq26.3	DXS102	(CA)n	
Xq27.1	DXS984	(CA)n	
Xq27.2	DXS292	(CA)n	
Xq27.3	DXS691	(CA)n	
Xq27.3	DXS998	(CA)n	
Xq27.3	FRAXA	(CCG)n	
Xq28	FRAXE	(GGC)n	
Xq28	DXS1113	(CA)n	
Xq28	DXS305	U6.2	TaqI
Xq28	DXS205	St.35-691	TaqI
Xq28	DXS52	VNTR	
Xq28	9120/9121	(CA)n	
Xq28	8345/8346	(CA)n	
Xq28	F8C5	(CA)n	
Xq28	DXS1108	(CA)n	

[a]Highly polymorphic minisatellite markers and RFLP markers used in the linkage analysis for MRX28 and MRX33. Information about primer sequences were taken from GDB except for the markers Lü1(STR9120/21) and Lü2(STR8345/46) [Wehnert et al., 1993].

Peripheral blood was cultured to give a high yield of early metaphases by inducing synchrony by blocking in S phase with amethopterin and then releasing with thymidine. Cells were G banded by using trypsin and Giemsa stain and analyzed by direct microscopy. Cultures were analyzed for the presence of fra(X) cells in low folate medium.

Linkage Analysis

Two-point and multipoint linkage analyses were performed with 40 polymorphic markers spread along the entire X chromosome by using version 5.1 of the program package LINKAGE [Lathrop et al., 1984] on a SUN-IPC workstation. A LOD score of >2 was regarded as significant to indicate linkage [Ott, 1991]. Genotypes were recoded to reduce the number of alleles where necessary to decrease memory requirements and computation time. Recoding was done by reducing alleles to those observed in the first generation of the family and adjusting those of younger individuals to preserve infomativity; equal allele frequencies were assumed for most of the highly polymorphic markers. Parameters for XLMR were set arbitrarily to a gene frequency of 1×10^{-4} and a mutation rate of 1.3×10^{-6}. Full penetrance in males and no penetrance in females were assumed because all obligate carrier females in the family showed normal intelligence. A phenocopy rate of 0.005 was chosen, allowing for other causes of mental impairment, although no risk factors such as severe birth complications existed for the affected relatives. Pairwise linkage analyses were performed by using the MLINK option of the linkage program package. Multipoint LOD scores were calculated by using LINKMAP, with overlapping sets of 5- and 6-point analyses.

RESULTS

Cytogenetic Investigations

Cytogenetic investigations of lymphocytes cultured in normal and low folate media were performed. Normal chromosomes with no detectable fragile sites at Xq27.3 or deletions were demonstrated for the affected males in both families. With the same methods, a normal female karyotype was obtained for the obligate carrier women.

DNA Analysis

In the affected males and in the obligate carrier women, repeat expansions in the fra(X)-A [Oostra and Verkerk, 1992] and fra(X)-E loci [Knight et al., 1993] were excluded by PCR and Southern blot analysis.

Linkage Analysis

Family 1 (MRX28). The gene causing mental retardation in this family was localized to a 14-cM interval distal to DXS1113 at Xq28. A maximum multipoint LOD score of 2.75 and a maximum 2-point LOD score of 2.53 were obtained at DXS52. The whole interval (DXS52 to telomere) showed LOD scores of >2, with a drop at STR8345/8346, which was excluded. Wide regions of the remaining X chromosome could be excluded formally with LOD scores of <2 (Fig. 2A).

Family 2 (MRX 33). The MRX gene in family 2 was localized to an interval of 30 cM at Xp11.4–Xp22.12 between DXS365 and MAOB. Maximal multipoint and 2-point LOD scores of 2.82 were obtained for markers at the DMD gene (STR49, DysII) and DXS1068. Negative LOD scores were obtained for almost all other regions of the X chromosome, and most regions could be formally excluded with LOD scores of <2 (Fig. 2B).

DISCUSSION

We have shown significant linkage for 2 families with nonsyndromic XLMR to 2 specific intervals on the X chromosome. Our results strongly support the existence of an MRX gene in Xq28 (MRX28) and an MRX gene in Xp11.4–22.12 (MRX33). The affected males in both families had no consistent clinical manifestations other than moderate mental retardation, although some had some anomalies. Obligate carriers in both families had normal intelligence. Conditions with similar manifestation (moderate mental retardation without further symptoms or heterozygote manifestation) are described in other XLMR families. The causative MRX genes in 4 families (MRX1, MRX4, MRX9, MRX17) were located to intervals other than MRX28 and MRX33 (Lubs, personal communication).

The XLMR gene in family 1 (MRX28) is located on Xq28, distal to the recombining marker DXS1113. The MRX genes for 3 other families with mental retardation as the only manifestation were mapped to Xq28. In the first family (MRX3), the linkage interval is defined by the marker DXS304 to qter, and the highest LOD scores were obtained with DXS52 [Gedeon et al., 1991]. In the second family, the linkage interval reaches from DXS52 to qter and has been reported [Hamel et al., 1995]. The clinical manifestations in both families are similar to family 1, with no consistent minor anomalies and no heterozygote manifestations. The linkage interval of a third family, now designated MRX25, is defined by the marker DXS297 and the telomeric end [Nordström et al., 1992]. The affected males of this family are severely retarded, and in contrast with our family the gene defect in this kindred results in heterozygote manifestations.

Two additional MRX families that were mapped to Xq28 can be differentiated by additional clinical findings. The affected males of family MRX6 show facial anomalies and short stature, and heterozygote females show mild mental retardation [Kondo et al., 1991]. In family MRX16, moderate mental retardation is combined with variable short stature, microcephaly, and neurological disturbances [Ronce et al., 1995].

The gene locus for family 2 (MRX33) is flanked by the recombining markers DXS365 and MAOB in Xp11.4–22.1. For many XLMR families, the underlying gene defect has been localized to the same chromosomal region. The corresponding intervals overlap at least partly with the gene locus MRX33 [Lubs, personal communication]. However, some family members show either additional characteristic anomalies [MRX12: Kerr et al., 1992] or additional neurological abnormalities besides mental impairment [MRX22: Passos-Bueno et al., 1993]. In the other families with only mental re-

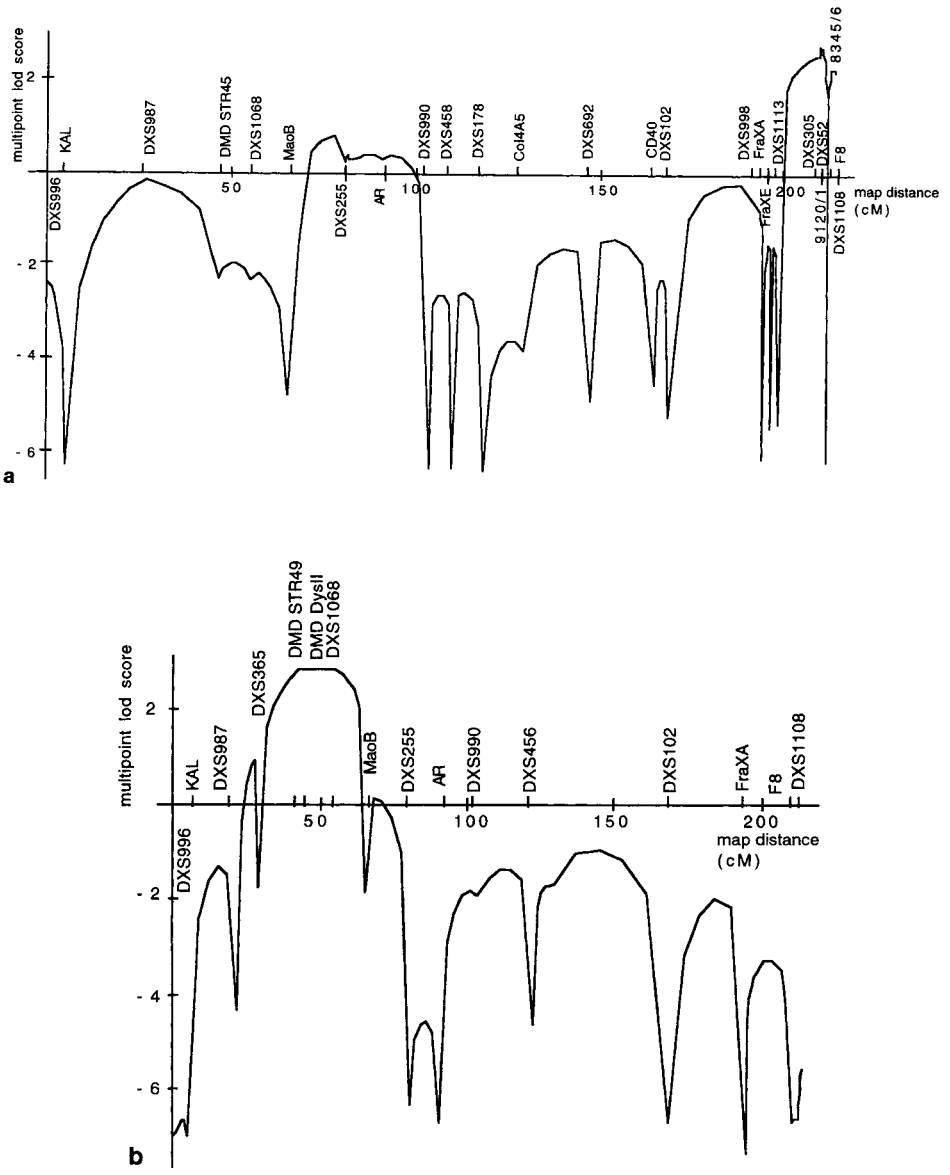

Fig. 2. Multipoint linkage analysis of XLMR and markers of the entire X chromosome in families MRX28 (**a**) and MRX33 (**b**). The location map indicates composite LOD scores for XLMR at various positions in a fixed marker map. The relative order and distances of the markers were taken from the CHLC Framework Map of the Chromosome X and from Donnelly et al. [1994]. For markers with unknown genetic distances, distances were chosen according to physical location and distances of reference markers in the region. The order of markers Lü1 (STR9120/21) and Lü2 (STR 8345/46) is chosen arbitrarily. DXS996 at the telomeric end of the short arm of the X chromosome is the reference point with a location of zero.

tardation, heterozygote manifestation was observed [MRX10 and MRX11: Kerr et al., 1992; MRX18: Gedeon et al., 1994; MRX19: Donnelly et al., 1994; MRX21: Kozak et al., 1993]. Only 2 families genetically linked to this region show a similar clinical phenotype to family MRX33 (moderate mental retardation, no consistent anomalies, no heterozygote manifestation). In a Dutch family, the underlying gene was localized to a region between the markers DXS987 and DMD3′. Only moderate mental impairment was diagnosed in the affected males [Hamel et al., 1995]. In a Belgian family, the determined genetic interval was flanked by the markers DXS989 and DMD-Dys. Further investigation showed a microdeletion for the marker DXS1218 within this interval [Raeymaekers et al., 1995]. The same gene might be involved in patients showing either isolated mental retardation or a combination with adrenal hypoplasia congenita [Ellison et al., 1993].

Clinical differences in the families can be caused by either allelic variability or genetic heterogeneity. Whether a single mental retardation locus is involved in the clinically similar families and even in the fami-

lies with different manifestations remains unresolved. The question of allelic or genetic heterogeneity can be resolved only by the isolation of the corresponding genes. Large-scale isolation of X-chromosomal brain-specific cDNAs will be a pivotal approach to provide XLMR candidate genes. Until then, further refinement of the known MRX loci will result in smaller candidate gene intervals.

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